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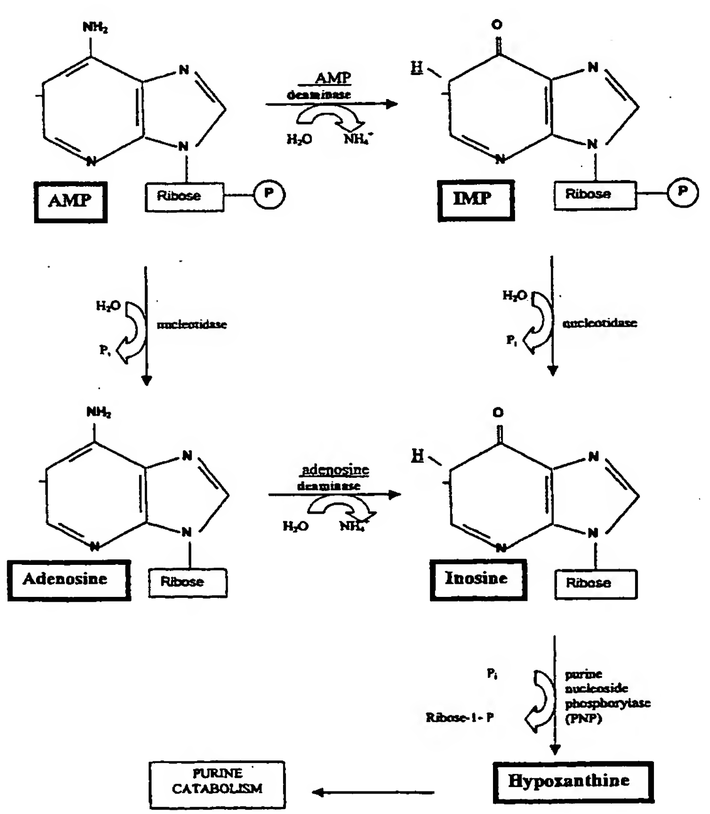

(57) Abstract: This invention relates to an isolated nucleic acid fragment encoding an AMP deaminase or adenosine deaminase, a transformed host cell comprising the nucleic acid fragment, and a transgenic plant comprising the nucleic acid fragment. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of an AMP or adenosine deaminase, and a chimeric gene comprising the isolated fragment in sense or antisense orientation. This invention further relates to a method for altering expression level of AMP deaminase or adenosine deaminase in a transformed host cell, and a method for evaluating a compound that affects the activity of an AMP deaminase or an adenosine deaminase.

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TITLE

PURINE METABOLISM GENES IN PLANTS

This application claims the benefit of U.S. Provisional Application No. 60/146473, filed July 30, 1999.

BACKGROUND OF THE INVENTION

Purines are components of DNA and RNA. Purine triphosphates also serve as chemical energy storage media for the cell. Regulation of purine synthesis and metabolism within the cell is critical to functions of all cells. Most mutations affecting nucleotide biosynthetic enzymes are lethal, although certain redundancy and salvage pathways may moderate the deleterious effects of some of these mutations.

Adenosine deaminase and AMP deaminase are two enzymes that convert adenosine to inosine. Mutations in these genes cause disruptions in the salvage and catabolism of adenosine and AMP. In humans these mutations may lead to death of white blood cells (lymphocytes) which in turn cause severe immunodeficiencies (Wilson et al. (1991) *Science 252*:1278-1284; Morgan and Anderson (1993) *Ann Rev Biochem 62*:191-217; Markert (1994) *Immunodeficiency 5*:141-157). Selective disruption of adenosine or AMP deaminase activities may be exploited in the identification and production of novel herbicides, insecticides, and fungicides.

SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising: (a) a first nucleotide encoding a polypeptide that is at least 35 amino acids in length and that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22, or (b) a second nucleotide sequence that is a complement of the first nucleotide sequence.

In a second embodiment, the isolated polynucleotide of the invention comprises a nucleotide having a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21.

In a third embodiment, this invention concerns an isolated polynucleotide comprising at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a member selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or a complement thereof.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns an isolated host cell comprising a chimeric gene or an isolated polynucleotide of the present invention. The host cell may be

eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing an isolated host cell, the process comprising either transforming or transfecting an isolated host cell with a suitable chimeric gene or isolated polynucleotide.

In a seventh embodiment, the invention concerns an isolated polypeptide that is at least 35 amino acids in length and that has at least 85% identity based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22.

In an eighth embodiment, the invention relates to a method of selecting an isolated polynucleotide that affects the expression level or activity of an AMP or adenosine deaminase in a host cell, preferably a plant cell. According to the invention, the method comprises: (a) constructing an isolated polynucleotide or an isolated chimeric gene of the present invention; (b) introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; (c) measuring the level of the AMP or adenosine deaminase polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and (d) selecting a suitable polynucleotide via comparing the level of the AMP or adenosine deaminase polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of the AMP or adenosine deaminase polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

In a ninth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of an AMP or adenosine deaminase polypeptide, preferably a plant AMP or adenosine deaminase polypeptide, comprising: synthesizing an oligonucleotide primer comprising at least 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or from a complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an AMP or adenosine deaminase amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of an AMP or adenosine deaminase comprising: probing a cDNA or genomic library with a labeled, isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with the isolated polynucleotide; and isolating the identified DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide of the present invention.

In a twelfth embodiment, this invention concerns a method for obtaining a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or an expression cassette comprising an isolated polynucleotide of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot plant cell, under conditions which allow expression of the polynucleotide in an amount sufficient to complement a null mutation.

In a thirteenth embodiment, this invention relates to a method of altering the level of expression of enzymes involved in purine metabolism in a host cell comprising: (a) transforming a host cell with a chimeric gene of the present invention; and (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene alters levels of the enzymes involved in purine metabolism in the transformed host cell.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an enzyme involved in purine metabolism, the method comprising the steps of: (a) transforming a host cell with a chimeric gene of the invention; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the enzyme in the transformed host cell; (c) optionally purifying the expressed enzyme by the transformed host cell; (d) treating the enzyme with a compound to be tested; and (e) comparing the activity of the enzymes that has been treated to the activity of an untreated enzyme, thereby selecting compounds with inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listings.

Figure 1 depicts the biochemical pathway leading to purine catabolism. The steps regulated by AMP deaminase and adenosine deaminase are noted.

Figure 2 shows a comparison of the amino acid sequences of corn, rice, soybean, and wheat AMP deaminases (SEQ ID NOs:14, 16, 18, and 20, respectively) and the *Arabidopsis thaliana* AMP deaminase (SEQ ID NO:23 [gi 7484807]).

Figure 3 shows a comparison of the amino acid sequence of the soybean adenosine deaminase (SEQ ID NO:12) to the *Escherichia coli* adenosine deaminase (SEQ ID NO:24 [gi 2506342]).

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a

substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Enzymes Involved in Purine Metabolism

AMP Deaminase	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Corn (*Zea mays*)	p0091.cqrav79r	1	2
Rice (*Oryza sativa*)	rsl1n.pk001.a12	3	4
Soybean (*Glycine max*)	sgs3c.pk001.m22	5	6
Wheat (*Triticum aestivum*)	wr1.pk0133.f5	7	8
Corn (*Zea mays*)	p0091.cqrav79r:fis	13	14
Rice (*Oryza sativa*)	rsl1n.pk001.a12:fis	15	16
Soybean (*Glycine max*)	sgs3c.pk001.m22:fis	17	18
Wheat (*Triticum aestivum*)	wr1.pk0133.f5:fis	19	20

Adenosine Deaminase	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Corn (*Zea mays*)	p0005.cbmfm83r p0005.cbmfm83rb	9	10
Soybean (*Glycine max*)	sls2c.pk003.f5	11	12
Soybean (*Glycine max*)	sls2c.pk003.f5	21	22

The Sequence Listing contains the standard one-letter codes for nucleotides and the three-letter codes for amino acids under the IUPAC-IUBMB (*Nucleic Acids Res. 13*:3021-3030 (1985); *Biochemical J. 219 (No. 2)*:345-373 (1984)). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822. The information disclosed in SEQ ID NOs:1-12 is contained in U.S. Provisional Application No. 60/146473, filed July 30, 1999.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, the terms "polynucleotide," "polynucleotide sequence," "nucleic acid sequence," and "nucleic acid fragment" or "isolated nucleic acid fragment" are used interchangeably. A polynucleotide may be a RNA or DNA that may be single- or double-stranded, and may contain synthetic, non-natural or altered nucleotide bases. A DNA polynucleotide may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 30 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 60 contiguous nucleotides

derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such sequences.

The term "isolated" polynucleotide or polypeptide refers to a molecule that is substantially free from other cellular molecules, such as other chromosomal and extrachromosomal DNA and RNA or other proteins, that normally accompany or interact with it as found in its naturally occurring environment. Isolated polynucleotides or polypeptides may be purified from a host cell in which they naturally occur. Conventional nucleic acid or protein purification methods are well known to skilled artisans. The term also embraces recombinant and chemically synthesized polynucleotides or polypeptides.

The term "recombinant" means, for example, that a nucleic acid sequence is made, or modified, by an artificial combination of two or more otherwise separated nucleic acid fragments by chemical synthesis or by genetic engineering techniques.

As used herein, "contig" refers to a contiguous nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share significant common or overlapping regions of sequence homology and can be assembled into a single contiguous nucleotide sequence, to form a "contig". Methods for comparing and

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment, or its transcripts, to alter gene expression patterns or levels, for example via gene silencing through antisense or co-suppression.

Substantially similar nucleic acid fragments may be obtained by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell.

A substantially similar nucleic acid fragment representing at least 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using substantially similar nucleic acid fragments,

that is, nucleic acid molecules representing less than the entire coding region of a gene, or having less than 100% sequence identity with the gene to be suppressed.

Substantially similar polypeptides refer to polypeptides having one or more amino acid substitutions, additions or deletions that do not significantly affect the function of the polypeptide. One skilled in the art recognizes that conservative substitutions, whereby a residue is substituted by another with like characteristics, result in polypeptides with substantially similar functions. Typical such substitutions are among Ala, Val, Leu and Ile; between Ser and Thr; between Asp and Glu; between Asn and Gln; and between Lys and Arg; or between Phe and Tyr. Substantially similar polypeptides may have several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids substituted, deleted, or added in any combination.

Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product.

Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of an AMP or adenosine deaminase.

It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms “substantially similar” and “corresponding substantially” are used interchangeably herein.

A method of selecting an isolated polynucleotide that affects the level of expression of a gene in a virus or in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise: constructing an isolated polynucleotide of the present invention or an chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell that expresses a native equivalent of the isolated polynucleotide; measuring the level of expression of the native gene in the host cell; and comparing the level of a polypeptide or enzyme activity in the host cell containing the

isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode, as determined by algorithms commonly employed by those skilled in this art. Suitable isolated polynucleotides of the present invention encode polypeptides that are at least about 70% identical, preferably at least about 80%, more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95% identical to the amino acid sequences reported herein.

Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 10, preferably 20, more preferably 30, still more preferably 50, more preferably at least 100, more preferably at least 150, still more preferably at least 200, and most preferably at least 250 amino acids.

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* *5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

Amino acid and nucleotide sequences can also be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools

that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers.

Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence.

The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of these sequences, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, it is contemplated that the instant invention encompasses complete sequences of the full-length molecules comprising the sequences as reported in the accompanying Sequence Listing, as well as substantial portions thereof.

"Codon degeneracy" refers to divergence in the genetic code permitting variations of the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein.

The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to code for a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable, and within the ordinary skill of the skilled artisan, to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks, which may be chemically synthesized, using procedures known to those skilled in the art. These building blocks are ligated or annealed to form larger nucleic acid fragments that may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized," as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid

fragments may be accomplished using well-established procedures. Automated chemical synthesis can be performed using one of a number of commercially available machines.

Chemical synthesis of polynucleotides allows the tailoring of a polynucleotide for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates that successful gene expression is more likely if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that code for a polypeptide molecule. A gene may also encode an RNA molecule. As generally understood, a gene includes regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence, as well as intervening sequences (introns) between individual coding segments (exons). "Native gene" refers to a gene with its native regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Indigenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign-gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes may be native genes inserted into a non-native organism, or they may be chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may

even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters." New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. **(Please use a more recent reference)** It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

The term "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or other aspects of gene expression. The polyadenylation signal is usually characterized by effecting the addition of a polyadenylate homopolymer to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript. When the primary transcript has undergone posttranscriptional processing, it becomes a mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptides by the cell. "cDNA" refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. "Sense-RNA" refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that may block the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the target gene, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence.

"Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments to form a single polynucleotide, so that the function of one fragment is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation. For example, a coding region could be linked to a promoter such that an sense or an antisense transcript is expressed. In addition, some regulatory elements can function in a orientation-independent manner.

The term "expression," as used herein, refers to the transcription of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

"Antisense inhibition" refers to the production of antisense RNA transcripts, which in turn suppress the expression of the target gene. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts, which suppress the expression of identical or substantially similar foreign or indigenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" of expression, or "altered expression," refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Null mutant," when used to refer to a cell, means that the cell either lacks the expression of a certain gene product or expresses a gene product which is inactive or does not have any detectable expected function.

"Mature protein" or the term "mature" when used in describing a protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed (need for functional reference?). "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in a plant cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53).

If the protein is to be directed to a vacuole, a vacuolar targeting signal (has not appeared previously) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Physiol. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, preferably resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of plant transformation methods include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) (any newer reference?) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Flevin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

"PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) first nucleotide sequence encoding a polypeptide of at least 30, preferably 60, still preferably 100, more preferably 150, still more

preferably 200, more preferably 250, more preferably 300, still preferably 350, still more preferably 400, still preferably 450, more preferably 500, more preferably 550, and most preferably 650 amino acids, having at least at least 30%, preferably 35%, still preferably 40%, more preferably 45%, still more preferably 50%, more preferably 55%, more preferably 60%, still preferably 65%, still more preferably 70%, still preferably 75%, more preferably 80%, more preferably 85%, and most preferably 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Preferably, the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. A particularly preferred polypeptide of the invention has an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 22.

This invention provides nucleic acid fragments encoding at least a portion of several AMP or adenosine deaminases. These nucleic acids have been isolated and identified by comparison of plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art.

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction and ligase chain reaction).

For example, genes encoding AMP or adenosine deaminases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. For example, one primer may be based on the sequence of the instantly disclosed polynucleotide molecules, the other primer may be based on polyA or the sequence on a vector, depending on the template used in the amplification.

Specifically, PCR may be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (Gibco BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 30 (preferably one of at least 40, most preferably one of at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such nucleotide sequences, may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention further relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a plant AMP or adenosine deaminases, comprising the steps of: synthesizing an first oligonucleotide primer comprising a nucleotide sequence of at least one of 30 (preferably at least one of 40, most preferably at least one of 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or the complement of such nucleotide sequences; synthesizing a suitable second oligonucleotide primer, and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of an AMP or adenosine deaminase.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing the entirety of portions of the instant amino acid sequences are synthesized. These peptides are

used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences of the synthetic peptides. These antibodies can then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention or an isolated polynucleotide of the invention. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast cells, bacterial cells, and plant cells. Examples of suitable viruses include (need list for written description).

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are overexpressed or their expression suppressed. This would alter the level of floral gene expression in those cells and/or flower development of transgenic plants comprising these polynucleotides.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes, and 3' non-coding sequences encoding transcription termination signals. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide (or chimeric gene) may be constructed. The skilled artisan readily recognizes that the choice of plasmid vector is dependent upon many factors, such as when the vector for protein expression, gene over-expression or suppression, in what type of host cell the vectors are propagated, and on the method that will be used to transform host plants.

The skilled artisan is well aware of the elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene.

The skilled artisan also recognizes that different transformation events may result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and that one may have to screen multiple transformation events to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

It may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate their secretion from the cell. Accordingly, the chimeric gene of the invention

may be further supplemented with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.100*:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants via co-suppression of antisense suppression.. A chimeric gene can be constructed by linking a gene or gene fragment encoding the polypeptide of interest in frame, either in the sense orientation or in the antisense orientation to suitable plant promoter sequences. The chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would most likely act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity, these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a plant breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to a specific plant tissue using tissue-specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing appropriate regulatory elements known to the skilled artisan. Once transgenic plants are obtained, it will be necessary to screen individual transgenic plants for those that most effectively display the desired phenotype. The appropriate screening method will generally be chosen on practical grounds and are within the skills of the ordinary artisan. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or by assaying the relevant enzyme activity. A preferred method allows large numbers of samples to be processed rapidly.

In another embodiment, the present invention concerns a polypeptide of at least 35 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in microbes, and is used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* or *in vitro*. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art and may be used for the instant invention. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded floral development proteins. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 7).

Additionally, the instant polypeptides can be used to design or identify herbicides that inhibit their enzyme activities. This is desirable because inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth or even plant death.

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping (*see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, (1996) Academic Press, pp. 319-346, and references cited therein) the genes that they compose, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes.

For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map.

In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individual plants representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is used to calculate the position of the instant nucleic acid sequence in a genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in, for example, Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter 4*:37-41. F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet. 7*:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res. 5*:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods for genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med. 11*:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics 16*:325-332), allele-specific ligation (Landegren et al. (1988) *Science 241*:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res. 18*:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet. 7*:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res. 17*:6795-6807).

For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a plant population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA 86*:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA 92*:8149-8153; Bensen et al. (1995) *Plant Cell 7*:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see

Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean, and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean, and Wheat

Library	Tissue	Clone
p0097	V9 7cm whorl section + ECB1, screened 1 B73+ECB1: 7-cm whorl section Growth conditions: field plots; these plants have been infested with ECB four times prior to harvest. Growth stage: unknown; V9 or V10	p0097.cqrav79r p0097.cqrav79r:fis
rsl1n*	Rice (Oryza sativa, YM) 15 day old seedling normalized	rsl1n.pk001.a12 rsl1n.pk001.a12:fis
sgs3c	Soybean Seeds 25 Hours After Germination	sgs3c.pk001.m22 sgs3c.pk001.m22:fis
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk0133.f5 wr1.pk0133.f5:fis
p0005	Immature Ear, two	p0005.cbmfm83r p0005.cbmfm83rb
sls2c	Soybean (Glycine max L., Manta) infected with *Sclerotinia sclerotiorum* mycelium.	sls2c.pk003.f5 sls2c.pk003.f5:fis

*This library was normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA templates are reacted with vector primed M13 forward and reverse oligonucleotides in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is

performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res. 22*:3765-3772). The *in vitro* transposition system places unique binding sites randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res. 11*:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred/Phrap (P. Green, University of Washington, Seattle). Phred/Phrap is a public domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (D. Gordon, University of Washington, Seattle).

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding AMP or adenosine deaminases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a

sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the GenBank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res. 25*:3389-3402.) against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 1. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

EXAMPLE 3

Characterization of cDNA Clones Encoding AMP Deaminase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to AMP deaminase from human [*Homo sapiens*](NCBI General Identifier No. gi644509) and yeast [*Saccharomyces cerevisiae*](NCBI General Identifier No. gi351916). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to AMP Deaminase

		BLAST pLog Score	
Clone	Status	gi644509	gi1351916
p0097.cqrav79r	EST	179.00	
rsl1n.pk001.a12	EST	175.00	
sgs3c.pk001.m22	EST		39.3
wr1.pk0133.f5	EST		22.4

The sequence of the entire cDNA insert in the clones listed in Table 3 was determined. The BLASTX search using the EST sequences from clones listed in Table 4 revealed similarity of the polypeptides encoded by the cDNAs to AMP deaminase from *Arabidopsis* [*Arabidopsis thaliana*](NCBI General Identifier No. gi 7484807). Shown in Table 4 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 4

BLAST Results for Sequences Encoding Polypeptides Homologous to AMP Deaminase

Clone	Status	BLAST pLog Score gi7484807
p0097.cqrav79r:fis	CGS	254.00
rsl1n.pk001.a12:fis	CGS	254.00
sgs3c.pk001.m22:fis	CGS	254.00
wr1.pk0133.f5:fis	FIS	254.00

Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:14, 16, 18 and 20, and the *Arabidopsis thaliana* sequence (SEQ ID NO:23). The data in Table 5 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 14, 16, 18 and 20, and the human (NCBI General Identifier No. gi 644509), yeast (NCBI General Identifier No. gi 351916), and *Arabidopsis thaliana* sequences (SEQ ID NO:23).

TABLE 5

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to AMP Deaminase

SEQ ID NO.	gi 644509	Percent Identity to gi 1351916	gi7484807
2	42.3%		
4	60.7%		
6		70.7%	
8		43.9%	
14			81.7%
16			82.3%
18			78.0%
20			82.6%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of an AMP deaminase. These sequences represent the first corn, rice, soybean, and wheat sequences encoding AMP deaminase known to Applicant.

EXAMPLE 4

Characterization of cDNA Clones Encoding Adenosine Deaminase

The BLASTX search using the EST sequences from clones listed in Table 6 revealed similarity of the polypeptides encoded by the cDNAs to adenosine deaminase from *Arabidopsis* [*Arabidopsis thaliana*] (NCBI General Identifier No. gi 4115949) and yeast (*Saccharomyces cerevisiae*) (NCBI General Identifier No. gi 1703166). Shown in Table 6 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 6

BLAST Results for Sequences Encoding Polypeptides Homologous to Adenosine Deaminase

		BLAST pLog Score	
Clone	Status	gi 4115949	gi 1703166
Contig of p0005.cbmfm83r p0005.cbmfm83rb	Contig	14.7	
sls2c.pk003.f5	EST		17.3

The sequence of the entire cDNA insert in the clones listed in Table 6 was determined. The BLASTX search using the CGS sequence from the soybean clone listed in Table 7 revealed similarity of the polypeptide encoded by the cDNA to adenosine deaminase from *Escherichia coli* (NCBI General Identifier No. gi 2506342). Shown in Table 7 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to Adenosine Deaminase

Clone	Status	BLAST pLog Score 2506342
sls2c.pk003.f5:fis	GCS	31.52

Figure 3 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:22 and the *Escherichia coli* sequence (SEQ ID NO:24). The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:10, 12, and 22, and the *Arabidopsis* (NCBI General Identifier No. gi 4115949), yeast (NCBI General Identifier No. gi 1703166), and *Escherichia coli* sequences (SEQ ID NO:24).

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Adenosine Deaminase

	Percent Identity to		
SEQ ID NO.	gi 4115949	gi 1703166	gi 2506342
10	71.7%		
12		67.6%	
22			25.8%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. *5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of an adenosine deaminase. These sequences represent the first soybean sequence encoding adenosine deaminase known to Applicant.

EXAMPLE 5

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli*

XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst AG, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad

Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* *8*:833-839).

EXAMPLE 6

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μL of a 60 mg/mL 1 μm gold particle suspension is added (in order): 5 μL DNA (1 μg/μL), 20 μl spermidine (0.1 M), and 50 μL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μL 70% ethanol and resuspended in 40 μL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the

retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 7

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% low melting agarose gel. Buffer and agarose contain 10 μg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs (NEB), Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene

encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 8

Evaluating Compounds for Their Ability to Inhibit the Activity of Enzymes Involved in Purine Metabolism

The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 7, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("$(His)_6$"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the

instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a $(His)_6$ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β–mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for AMP deaminase are presented by Meyers et al. (1989) *Biochem 28*:8734-8743. Assays for adenosine deaminase are presented by Pelcher in U.S. Patent No. 5,474,929.

What is claimed is:

1. An isolated polynucleotide:

(a) a first nucleotide sequence encoding a polypeptide of at least 35 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a member selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22; and

(b) a second nucleotide sequence comprising a complement of the first nucleotide sequence.

2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence comprises of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21.

3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is DNA.

4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is RNA.

5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

6. An isolated host cell comprising the chimeric gene of Claim 5.

7. A host cell comprising an isolated polynucleotide of Claim 1.

8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of a yeast cell, a bacterial cell, and a plant cell.

9. A virus comprising the isolated polynucleotide of Claim 1.

10. A polypeptide of at least 35 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 12, 14, 16, 18, 20, and 22.

11. A method of selecting a polynucleotide molecule that affects the level of expression of an AMP or adenosine deaminase in a plant cell, the method comprising:

(a) providing a polynucleotide molecule comprising at least 30 contiguous nucleotides derived from the isolated polynucleotide of Claim 1;

(b) introducing the polynucleotide molecule into a plant cell that expresses an AMP or adenosine deaminase; and

(c) comparing the level of AMP or adenosine deaminase in the plant cell containing the isolated polynucleotide with the level of AMP or adenosine deaminase in a plant cell that does not contain the isolated polynucleotide.

12. The method of Claim 11 wherein the polynucleotide molecule is an isolated polynucleotide of Claim 1.

13. The method of Claim 11 wherein the polynucleotide molecule is an isolated polynucleotide selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21.

14. A method of obtaining a nucleic acid fragment encoding an AMP or adenosine deaminases polypeptide, the method comprising the steps of:

(a) providing an oligonucleotide primer at least 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or derived from a complement of the nucleotide sequences; and

(b) amplifying a nucleic acid sequence using the oligonucleotide primer.

15. A method of obtaining a nucleic acid fragment encoding an AMP or adenosine deaminases polypeptide, the method comprising the steps of:

(a) probing a cDNA or genomic library with a nucleotide probe comprising at least 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 11, 13, 15, 17, 19, and 21, or derived from a complement of such nucleotide sequences;

(b) identifying a clone that hybridizes with the probe; and

(c) isolating the clone.

16. A composition comprising the isolated polynucleotide of Claim 1.

17. A composition comprising the isolated polypeptide of Claim 10.

18. A transformed plant cell comprising the chimeric gene of Claim 5.

19. A method for producing a transformed cell comprising transforming a cell with the chimeric gene of Claim 5.

20. The method of Claim 19 wherein the cell is a plant cell.

21. The method of Claim 20 wherein the plant cell is a monocot plant cell.

22. The method of Claim 20 wherein the plant cell is a dicot plant.

23. A method of altering the expression level an AMP deaminase or an adenosine deaminase in a host cell comprising:

(a) providing a transformed host cell comprising the chimeric gene of Claim 5; and

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene alters the expression levels of the AMP deaminase or the adenosine deaminase in the transformed host cell.

24. A method for evaluating a compound for its ability to inhibit the activity of an AMP deaminase or an adenosine deaminase, the method comprising:

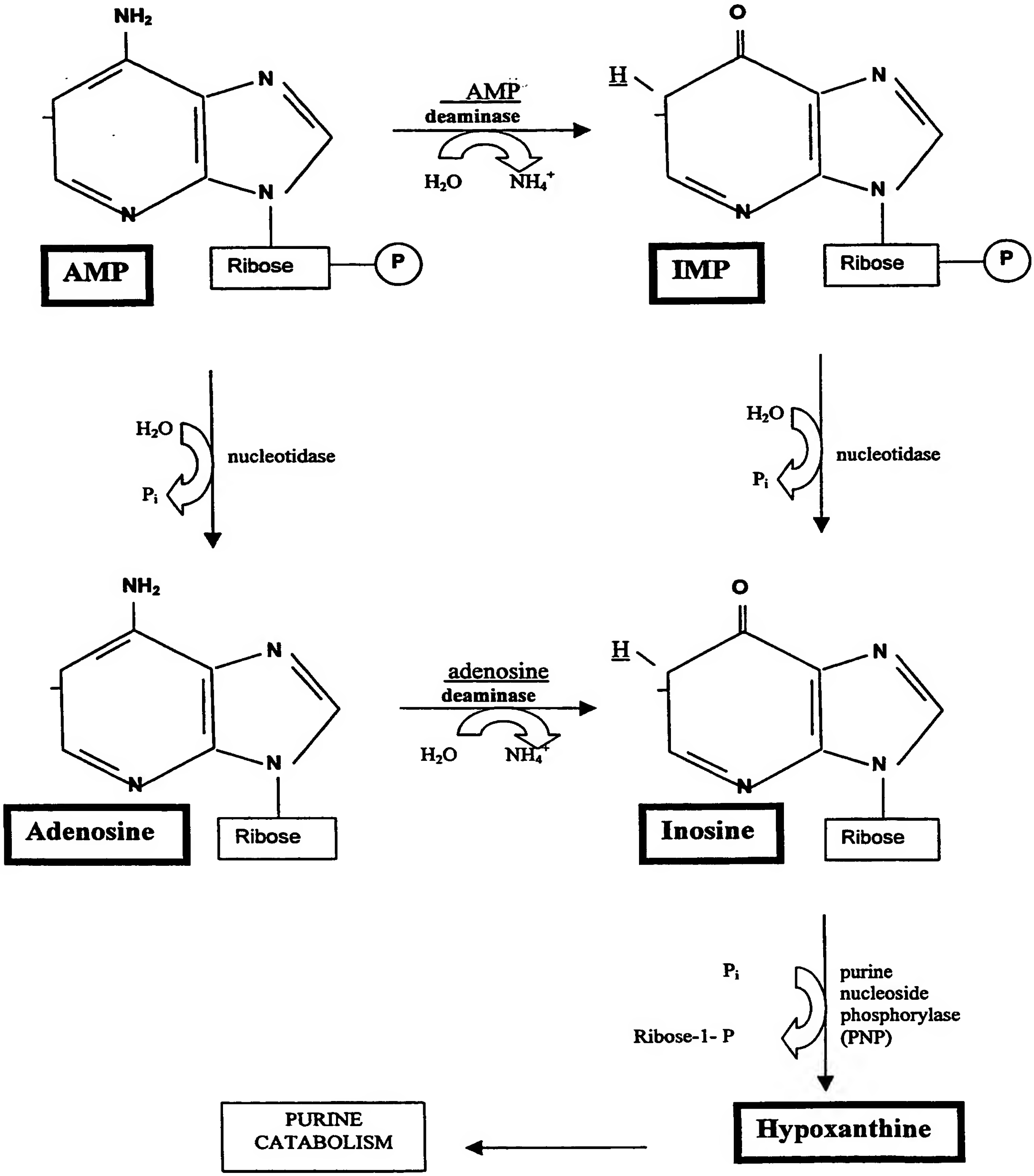
(a) providing a transformed host cell with a chimeric gene comprising a nucleic acid fragment encoding an AMP deaminase or an adenosine deaminase;

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene, wherein the expression produces an AMP deaminase or an adenosine deaminase;

(c) optionally purifying the AMP deaminase or adenosine deaminase produced;

(d) treating the AMP deaminase or adenosine deaminase polypeptide with a compound to be tested; and

(e) determining the inhibitory effect of the compound tested by comparing the activity of the AMP deaminase or adenosine deaminase polypeptide that has been treated to the activity of an untreated enzyme.



NH2
N
N
N
AMP
Ribose
P
AMP
deaminase
H2O
NH4+
O
H
N
N
N
IMP
Ribose
P
H2O
Pi
nucleotidase
H2O
Pi
nucleotidase
NH2
N
N
N
Adenosine
Ribose
adenosine
deaminase
H2O
NH4+
O
H
N
N
N
Inosine
Ribose
Pi
Ribose-1- P
purine
nucleoside
phosphorylase
(PNP)
Hypoxanthine
PURINE
CATABOLISM

FIGURE 2

Comparison of AMP Deaminase Polypeptides From Corn, Rice, Soybean, and Wheat (SEQ ID NOs:14, 16, 18, and 20) and *Arabidopsis thaliana*

```
SEQ ID NO:14   ---------------------------------------------------------------------------------EIPTADEIE
SEQ ID NO:16   MPKTMPVSLLTKWND--IGSDPLPGKASQNGDTKPVPSTNMIRSQSATGSLHGAQHNPVAADILRKEPEHETFSRINITAVETPSPDEIE
SEQ ID NO:18   LDTTYLHANGTGGPEGKIPFEPLPNHVNANGEQMAITPS-MIRSHSVSGDLHGVQPDPIAADILRKEPEHETFTRLRITPLEAPSPDEIE
SEQ ID NO:20   ------------------------------------------------------------------------------------------
gi 7484807     M-----ICL------------------------------------------------------------------------EVPTSDEVE

SEQ ID NO:14   VFKILXKCLELRDSYLFREEVAPWEKEVINDPCTPKPNPNPFTYVPEPKSEHVFQTVDGVIHVYADKDCTESIYPVADATTFFTDLHYIL
SEQ ID NO:16   AYKVLQKCLELREKYMFREEVAPWEKEIITDPSTPKPNPNPFYYEQQTKTEHHFEMVDGVIHVYPNKDAKERIYPVADATTFFTDMHYIL
SEQ ID NO:18   AYVVLQECLEMRKRYVFREAVAPWDKEVISDPSTPKPNPDPFLYIPEGNSDHYFEMQDGVIRVYPDRDAKEELFPVADATTFFTDLHHLL
SEQ ID NO:20   -----R-C----------------------------------------------------------------------------------
gi 7484807     AYKCLQECLELRKRYVFQETVAPWEKEVISDPSTPKPNTEPFAHYPQGKSDHCFEMQDGVVHVFANKDAKEDLFPVADATAFFTDLHHVL

SEQ ID NO:14   RVTAAGNTRTVCHNRLNLLEHKFKFHLMLNADREFLAQKTAPHRDFYNVRKVDTHVHHSACMNQKHLLRFIKSKLRKEPDEVVIFRDGTY
SEQ ID NO:16   RVLAAGDIRTVCYKRLNLLEQKFNLHLMVNADRELLAQKAAPHRDFYNVRKVDTHVHHSACMNQKHLLRFIKSKLRKEPDEVVIFRDGTY
SEQ ID NO:18   RVIAAGNIRTLCHHRLNLLEQKFNLHLMLNADREFLAQKSAPHRDFYNVRKVDTHVHHSACMNQKHLLRFIKSKLRKEPDEVVIFRDGTY
SEQ ID NO:20   ---------------------KF-------------------------------------------------------------------
gi 7484807     KVIAAGNIRTLCHRRLVLLEQKFNLHLMLNADKEFLAQKSAPHRDFYNVRKVDTHVHHSACMNQKHLLRFIKSKLRKEPDEVVIFRDGTY

SEQ ID NO:14   MTLKEVFESLDLTGYDLNVDLLDVHADKSTFHRFDKFNLKYNPCGQSRLREIFLKQDNLIQGRFLAELTKQVFSDLSASKYQMAEYRISI
SEQ ID NO:16   LTLKEVFESLDLTGYDLNVDLLDVHADKSTFHRFDKFNLKYNPCGQSRLREIFLKQDNLIQGRFLAELTKEVFSDLEASKYQMAEYRISI
SEQ ID NO:18   LTLEEVFKSLDLSGYDLNVDLLDVHADKSTFHRFDKFNLKYNPCGQSRLREIFLKQDNLIQGRFLGELTKQVFSDLAASKYQMAEYRISI
SEQ ID NO:20   ------------------------------------------------------------------------FSDLNASKYQMAEYRISI
gi 7484807     LTLREVFESLDLTGYDLNVDLLDVHADKSTFHRFDKFNLKYNPCGQSRLREIFLKQDNLIQGRFLGEITKQVFSDLEASKYQMAEYRISI

SEQ ID NO:14   YGRKQSEWDQLASWIVNNELHSGNVVWLVQIPRLYNVYKEMGIVTSFQNLLDNIFVPLFEVTIDPASHPQLHVFLKQVVGLDLVDDESKP
SEQ ID NO:16   YGRKKSEWDQMASWIVNNELYSENVVWLIQIPRIYNVYREMGTINSFQNLLDNIFLPLFEVTVDPASHPQLHVFLQQVVGLDLVDDESKP
SEQ ID NO:18   YGRKQSEWDQLASWIVNNDLYSENVVWLIQLPRLYNVYKEMGIVTSFQNMLDNIFIPLFEVTVNPDSHPQLHVFLKQVVGLDLVDDESKP
SEQ ID NO:20   YGRKQSEWDQLASWIVNNELYSENVVWLIQIPRLYNVYQQMGIVTSFQNLLDNIFLPLFEVTIDPASHPQLHVFLKQVVGLDLVDDESKP
gi 7484807     YGRKMSEWDQLASWIVNNDLYSENVVWLIQLPRLYNIYKDMGIVTSFQNILDNIFIPLFEATVDPDSHPQLHVFLKQVVGFDLVDDESKP
```

FIGURE 2 (continued)

SEQ ID NO:14	ERRPTKHMPTPEQWTNVFNPAFSYYAYYCYANLFTLNKLRESKGMTTIKFRPHAGEAGDVDHLAATFLLCHNISHGINLRKSPVLQYLYY
SEQ ID NO:16	ERRPTKHMPTPEQWTNVFNPAYAYYVYYCYANLYTLNKLRESKGMTTIKLRPHCGEAGDIDHLAAAFLTSHNIAHGVNLKKSPVLQYLYY
SEQ ID NO:18	ERRPTKHMPTPEQWTNVFNPAFSYYVYYCYANLYTLNKLRESKGMTTIKFRPHSGEAGDIDHLAATFLTAHNIAHGINLKKSPVLQYLYY
SEQ ID NO:20	ERRPTKHMPTPEEWTNVFNPAFSYYAYYCYANLYTLNKLRESKGMNTIKFRPHAGEAGDVDHLAATFLLCHSISHGINLRKSPVLQYLYY
gi 7484807	ERRPTKHMPTPAQWTNAFNPAFSYYVYYCYANLYVLNKLRESKGMTTITLRPHSGEAGDIDHLAATFLTCHSIAHGINLRKSPVLQYLYY
SEQ ID NO:14	LGQIGLAMSPLSNNSLFLDYHRNPFPTFFQRGLNVSLSTDDPLQIHLTKEPLVEEYSIAASLWKLSSCDLCEIARNSVYQSGFSHALKAH
SEQ ID NO:16	LAQIGLAMSPLSNNSLFIDYHRNPFPTFFLRGLNVSLSTDDPLQIHLTKEPLVEEYSIAASLWKLSSCDLCEIARNSVYQSGFSHRLKSH
SEQ ID NO:18	LAQIGLAMSPLSNNSLFLDYHRNPFPMFFLRGLNVSLSTDDPLQIHLTKEPLVEEY----------------------------------
SEQ ID NO:20	LGQIGLAMSPLSNNSLFLDYHRNPFPMFFQRGLNVSLSTDDPLQIHLTKEPLVEEYSIAASLWKLSSCDLCEIARNSVYQSGFSHALKAH
gi 7484807	LAQIGLAMSPLSNNSLFLDYHRNPFPVFFLRGLNVSLSTDDPLQIHLTKEPLVEEYSIAASVWKLSACDLCEIARNSVYQSGFSHALKSH
SEQ ID NO:14	WIGKNYFKRGPAGNDIHRTNVPHIRVQFREMIWRNEMKLVYSDNEILIPDELDL
SEQ ID NO:16	WIGRNYYKRGHDGNDIHQTNVPHIRIEFRHTIWKEEMELIHLRN-VDIPEEIDR
SEQ ID NO:18	--
SEQ ID NO:20	WIGKNYYKRGPSGNDIHRTNVPTIRIEFRDLIWRDEMQLVYLNNVIL-PDEVDQ
gi 7484807	WIGKDYYKRGPDGNDIHKTNVPHIRVEFRDTVWNE----IYL---FFTQVNFSL

FIGURE 3

Comparison of Adenosine Deaminase Polypeptides From Soybean (SEQ ID NO:22) and *E. coli*

```
SEQ ID NO:22    MCGENMKQFLKELPKCEHHIHIEGSLSPALLFELAKTNNIALPDSAADASFKSPQELESRYERFTSLNDFLHYYYIGMSVLINPADYESL
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Tyr Gln Ser Gly Phe Ser His Ala Leu Lys Ala His Trp Ile Gly Lys
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Asn Tyr Phe Lys Arg Gly Pro Ala Gly Asn Asp Ile His Arg Thr Asn
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Val Pro His Ile Arg Val Gln Phe Arg Glu Met Ile Trp Arg Asn Glu
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Arg Asp Phe Tyr Asn Val Arg Lys Val Asp Thr His Val His His Ser
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Ala Cys Met Asn Gln Lys His Leu Leu Arg Phe Ile Lys Ser Lys Leu
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Arg Lys Glu Pro Asp Glu Val Val Ile Phe Arg Asp Gly Thr Tyr Leu
65                  70                  75                  80

Thr Leu Lys Glu Val Phe Glu Ser Leu Asp Leu Thr Gly Tyr Asp Leu
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Asn Val Val Trp Leu Ile Gln Ile Pro Arg Ile Tyr Asn Val Tyr Arg
195 200 205

Glu Met Gly Thr Ile Asn Ser Phe Gln Asn Leu Leu Asp Asn Ile Phe
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Leu Pro Leu Phe Glu Val Thr Val Asp Pro Ala Ser His Pro Gln Leu
225 230 235 240

His Val Phe Leu Gln Gln Val Val Gly Leu Asp Leu Val Asp Asp Glu
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Ser Lys Pro Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro Glu Gln
260 265 270

Trp Thr Asn Val Phe Asn Pro Ala Tyr Ala Tyr Tyr Val Tyr Tyr Cys
275 280 285

Tyr Ala Asn Leu Tyr Thr Leu Asn Lys Leu Arg Glu Ser Lys Gly Met
290 295 300

Thr Thr Ile Lys Leu Arg Pro His Cys Gly Glu Ala Gly Asp Ile Asp
305 310 315 320

His Leu Ala Ala Ala Phe Leu Thr Ser His Asn Ile Ala His Gly Val
325 330 335

Asn Leu Lys Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu Ala Gln
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Ile Gly Leu Ala Met Ser Pro Leu Ser Asn Asn Ser Met Phe Ile Asp
355 360 365

Tyr His Arg Asn Pro Phe Pro Thr Phe Phe Leu Arg Gly Leu Asn Val
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Ser Leu Ser Thr Asp Asp Pro Leu Gln Ile His Leu Thr Lys Glu Pro
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Cys Asp Leu Cys Glu Ile Ala Arg Asn Ser Val Tyr Gln Ser Gly Phe
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Ser His Arg Leu Lys Ser His Trp Ile Gly Arg Asn Tyr Tyr Lys Arg
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Gly His Asp Gly Asn Asp Ile His Gln Thr Asn Val Pro His Ile Arg
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His Val His His Ser Ala Cys Met Asn Gln Lys His Leu Leu Arg Phe
35 40 45

Ile Lys Ser Lys Leu Arg Lys Glu Pro Asp Glu Val Val Ile Phe Arg
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Asp Gly Thr Tyr Leu Thr Leu Glu Glu Val Phe Lys Ser Leu Asp Leu
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35 40 45

Arg Leu Tyr Asn Val Tyr Gln Xaa Lys Trp Gly Leu Leu His His Phe
50 55 60

Lys Asn Phe Leu Gly Gln His Phe Pro Pro Pro Val Trp Arg Leu Leu
65 70 75 80

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Xaa Ser Ser Ser His Pro Ala Leu His Val Phe Pro Lys Ala Gly Leu
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Trp Glu Lys Glu Val Ile Asn Asp Pro Cys Thr Pro Lys Pro Asn Pro
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Tyr Ile Leu Arg Val Thr Ala Ala Gly Asn Thr Arg Thr Val Cys His
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Asn Arg Leu Asn Leu Leu Glu His Lys Phe Lys Phe His Leu Met Leu
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Asn Ala Asp Arg Glu Phe Leu Ala Gln Lys Thr Ala Pro His Arg Asp
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Phe Tyr Asn Val Arg Lys Val Asp Thr His Val His His Ser Ala Cys
145                 150                 155                 160

Met Asn Gln Lys His Leu Leu Arg Phe Ile Lys Ser Lys Leu Arg Lys
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Glu Pro Asp Glu Val Val Ile Phe Arg Asp Gly Thr Tyr Met Thr Leu
            180                 185                 190

Lys Glu Val Phe Glu Ser Leu Asp Leu Thr Gly Tyr Asp Leu Asn Val
        195                 200                 205

Asp Leu Leu Asp Val His Ala Asp Lys Ser Thr Phe His Arg Phe Asp
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Lys Phe Asn Leu Lys Tyr Asn Pro Cys Gly Gln Ser Arg Leu Arg Glu
225                 230                 235                 240

Ile Phe Leu Lys Gln Asp Asn Leu Ile Gln Gly Arg Phe Leu Ala Glu
                245                 250                 255

Leu Thr Lys Gln Val Phe Ser Asp Leu Ser Ala Ser Lys Tyr Gln Met
            260                 265                 270

Ala Glu Tyr Arg Ile Ser Ile Tyr Gly Arg Lys Gln Ser Glu Trp Asp
        275                 280                 285

Gln Leu Ala Ser Trp Ile Val Asn Asn Glu Leu His Ser Gly Asn Val
    290                 295                 300

Val Trp Leu Val Gln Ile Pro Arg Leu Tyr Asn Val Tyr Lys Glu Met
305                 310                 315                 320

Gly Ile Val Thr Ser Phe Gln Asn Leu Leu Asp Asn Ile Phe Val Pro
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Leu Phe Glu Val Thr Ile Asp Pro Ala Ser His Pro Gln Leu His Val
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Phe Leu Lys Gln Val Val Gly Leu Asp Leu Val Asp Asp Glu Ser Lys
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Pro Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro Glu Gln Trp Thr
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Ile Lys Phe Arg Pro His Ala Gly Glu Ala Gly Asp Val Asp His Leu
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Ala Ala Thr Phe Leu Leu Cys His Asn Ile Ser His Gly Ile Asn Leu
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Arg Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu Gly Gln Ile Gly
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Leu Ala Met Ser Pro Leu Ser Asn Asn Ser Leu Phe Leu Asp Tyr His
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Arg Asn Pro Phe Pro Thr Phe Phe Gln Arg Gly Leu Asn Val Ser Leu
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Ser Thr Asp Asp Pro Leu Gln Ile His Leu Thr Lys Glu Pro Leu Val
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Glu Glu Tyr Ser Ile Ala Ala Ser Leu Trp Lys Leu Ser Ser Cys Asp
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Leu Cys Glu Ile Ala Arg Asn Ser Val Tyr Gln Ser Gly Phe Ser His
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Ala Leu Lys Ala His Trp Ile Gly Lys Asn Tyr Phe Lys Arg Gly Pro
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Ala Gly Asn Asp Ile His Arg Thr Asn Val Pro His Ile Arg Val Gln
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Leu His Gly Ala Gln His Asn Pro Val Ala Ala Asp Ile Leu Arg Lys
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Thr Pro Ser Pro Asp Glu Ile Glu Ala Tyr Lys Val Leu Gln Lys Cys
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Leu Glu Leu Arg Glu Lys Tyr Met Phe Arg Glu Glu Val Ala Pro Trp
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Glu Lys Glu Ile Ile Thr Asp Pro Ser Thr Pro Lys Pro Asn Pro Asn
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Pro Phe Tyr Tyr Glu Gln Gln Thr Lys Thr Glu His His Phe Glu Met
    130                 135                 140

Val Asp Gly Val Ile His Val Tyr Pro Asn Lys Asp Ala Lys Glu Arg
145                 150                 155                 160

Ile Tyr Pro Val Ala Asp Ala Thr Thr Phe Phe Thr Asp Met His Tyr
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Ile Leu Arg Val Leu Ala Ala Gly Asp Ile Arg Thr Val Cys Tyr Lys
            180                 185                 190

Arg Leu Asn Leu Leu Glu Gln Lys Phe Asn Leu His Leu Met Val Asn
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Ala Asp Arg Glu Leu Leu Ala Gln Lys Ala Ala Pro His Arg Asp Phe
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Tyr Asn Val Arg Lys Val Asp Thr His Val His His Ser Ala Cys Met
225                 230                 235                 240

Asn Gln Lys His Leu Leu Arg Phe Ile Lys Ser Lys Leu Arg Lys Glu
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Pro Asp Glu Val Val Ile Phe Arg Asp Gly Thr Tyr Leu Thr Leu Lys
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Glu Val Phe Glu Ser Leu Asp Leu Thr Gly Tyr Asp Leu Asn Val Asp
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Leu Leu Asp Val His Ala Asp Lys Ser Thr Phe His Arg Phe Asp Lys
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Phe Asn Leu Lys Tyr Asn Pro Cys Gly Gln Ser Arg Leu Arg Glu Ile
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Phe Leu Lys Gln Asp Asn Leu Ile Gln Gly Arg Phe Leu Ala Glu Leu
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Thr Lys Glu Val Phe Ser Asp Leu Glu Ala Ser Lys Tyr Gln Met Ala
            340                 345                 350

Glu Tyr Arg Ile Ser Ile Tyr Gly Arg Lys Lys Ser Glu Trp Asp Gln
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Met Ala Ser Trp Ile Val Asn Asn Glu Leu Tyr Ser Glu Asn Val Val
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Leu Gln Gln Val Val Gly Leu Asp Leu Val Asp Asp Glu Ser Lys Pro
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Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro Glu Gln Trp Thr Asn
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Val Phe Asn Pro Ala Tyr Ala Tyr Tyr Val Tyr Tyr Cys Tyr Ala Asn
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Ala Ala Phe Leu Thr Ser His Asn Ile Ala His Gly Val Asn Leu Lys
        515                 520                 525

Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu Ala Gln Ile Gly Leu
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Ala Met Ser Pro Leu Ser Asn Asn Ser Leu Phe Ile Asp Tyr His Arg
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Asn Pro Phe Pro Thr Phe Phe Leu Arg Gly Leu Asn Val Ser Leu Ser
                565                 570                 575

Thr Asp Asp Pro Leu Gln Ile His Leu Thr Lys Glu Pro Leu Val Glu
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Glu Tyr Ser Ile Ala Ala Ser Leu Trp Lys Leu Ser Ser Cys Asp Leu
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Cys Glu Ile Ala Arg Asn Ser Val Tyr Gln Ser Gly Phe Ser His Arg
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Leu Lys Ser His Trp Ile Gly Arg Asn Tyr Tyr Lys Arg Gly His Asp
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Gly Asn Asp Ile His Gln Thr Asn Val Pro His Ile Arg Ile Glu Phe
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Gln Met Ala Ile Thr Pro Ser Met Ile Arg Ser His Ser Val Ser Gly
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Asp Leu His Gly Val Gln Pro Asp Pro Ile Ala Ala Asp Ile Leu Arg
    50                  55                  60

Lys Glu Pro Glu His Glu Thr Phe Thr Arg Leu Arg Ile Thr Pro Leu
65                  70                  75                  80

Glu Ala Pro Ser Pro Asp Glu Ile Glu Ala Tyr Val Val Leu Gln Glu
                85                  90                  95

Cys Leu Glu Met Arg Lys Arg Tyr Val Phe Arg Glu Ala Val Ala Pro
            100                 105                 110

Trp Asp Lys Glu Val Ile Ser Asp Pro Ser Thr Pro Lys Pro Asn Pro
        115                 120                 125

Asp Pro Phe Leu Tyr Ile Pro Glu Gly Asn Ser Asp His Tyr Phe Glu
    130                 135                 140

Met Gln Asp Gly Val Ile Arg Val Tyr Pro Asp Arg Asp Ala Lys Glu
145                 150                 155                 160

Glu Leu Phe Pro Val Ala Asp Ala Thr Thr Phe Phe Thr Asp Leu His
                165                 170                 175

His Leu Leu Arg Val Ile Ala Ala Gly Asn Ile Arg Thr Leu Cys His
            180                 185                 190

His Arg Leu Asn Leu Leu Glu Gln Lys Phe Asn Leu His Leu Met Leu
        195                 200                 205

Asn Ala Asp Arg Glu Phe Leu Ala Gln Lys Ser Ala Pro His Arg Asp
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Phe Tyr Asn Val Arg Lys Val Asp Thr His Val His His Ser Ala Cys
225                 230                 235                 240

Met Asn Gln Lys His Leu Leu Arg Phe Ile Lys Ser Lys Leu Arg Lys
                245                 250                 255

Glu Pro Asp Glu Val Val Ile Phe Arg Asp Gly Thr Tyr Leu Thr Leu
            260                 265                 270

Glu Glu Val Phe Lys Ser Leu Asp Leu Ser Gly Tyr Asp Leu Asn Val
        275                 280                 285

Asp Leu Leu Asp Val His Ala Asp Lys Ser Thr Phe His Arg Phe Asp
    290                 295                 300

Lys Phe Asn Leu Lys Tyr Asn Pro Cys Gly Gln Ser Arg Leu Arg Glu
305                 310                 315                 320

Ile Phe Leu Lys Gln Asp Asn Leu Ile Gln Gly Arg Phe Leu Gly Glu
                325                 330                 335

Leu Thr Lys Gln Val Phe Ser Asp Leu Ala Ala Ser Lys Tyr Gln Met
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Ala Glu Tyr Arg Ile Ser Ile Tyr Gly Arg Lys Gln Ser Glu Trp Asp
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Gln Leu Ala Ser Trp Ile Val Asn Asn Asp Leu Tyr Ser Glu Asn Val
    370                 375                 380

Val Trp Leu Ile Gln Leu Pro Arg Leu Tyr Asn Val Tyr Lys Glu Met
385                 390                 395                 400

Gly Ile Val Thr Ser Phe Gln Asn Met Leu Asp Asn Ile Phe Ile Pro
                405                 410                 415

Leu Phe Glu Val Thr Val Asn Pro Asp Ser His Pro Gln Leu His Val
            420                 425                 430

Phe Leu Lys Gln Val Val Gly Leu Asp Leu Val Asp Asp Glu Ser Lys
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Pro Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro Glu Gln Trp Thr
    450                 455                 460

Asn Val Phe Asn Pro Ala Phe Ser Tyr Tyr Val Tyr Tyr Cys Tyr Ala
465                 470                 475                 480

Asn Leu Tyr Thr Leu Asn Lys Leu Arg Glu Ser Lys Gly Met Thr Thr
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Ile Lys Phe Arg Pro His Ser Gly Glu Ala Gly Asp Ile Asp His Leu
            500                 505                 510

Ala Ala Thr Phe Leu Thr Ala His Asn Ile Ala His Gly Ile Asn Leu
        515                 520                 525

Lys Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu Ala Gln Ile Gly
    530                 535                 540

Leu Ala Met Ser Pro Leu Ser Asn Asn Ser Leu Phe Leu Asp Tyr His
545                 550                 555                 560

Arg Asn Pro Phe Pro Met Phe Phe Leu Arg Gly Leu Asn Val Ser Leu
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Glu Glu Tyr
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<212> PRT
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Leu Ala Ser Trp Ile Val Asn Asn Glu Leu Tyr Ser Glu Asn Val Val
35 40 45

Trp Leu Ile Gln Ile Pro Arg Leu Tyr Asn Val Tyr Gln Gln Met Gly
50 55 60

Ile Val Thr Ser Phe Gln Asn Leu Leu Asp Asn Ile Phe Leu Pro Leu
65 70 75 80

Phe Glu Val Thr Ile Asp Pro Ala Ser His Pro Gln Leu His Val Phe
85 90 95

Leu Lys Gln Val Val Gly Leu Asp Leu Val Asp Asp Glu Ser Lys Pro
100 105 110

Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro Glu Glu Trp Thr Asn
115 120 125

Val Phe Asn Pro Ala Phe Ser Tyr Tyr Ala Tyr Tyr Cys Tyr Ala Asn
    130   135   140

Leu Tyr Thr Leu Asn Lys Leu Arg Glu Ser Lys Gly Met Asn Thr Ile
145   150   155   160

Lys Phe Arg Pro His Ala Gly Glu Ala Gly Asp Val Asp His Leu Ala
                165   170   175

Ala Thr Phe Leu Leu Cys His Ser Ile Ser His Gly Ile Asn Leu Arg
            180   185   190

Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu Gly Gln Ile Gly Leu
        195   200   205

Ala Met Ser Pro Leu Ser Asn Asn Ser Leu Phe Leu Asp Tyr His Arg
    210   215   220

Asn Pro Phe Pro Met Phe Phe Gln Arg Gly Leu Asn Val Ser Leu Ser
225   230   235   240

Thr Asp Asp Pro Leu Gln Ile His Leu Thr Lys Glu Pro Leu Val Glu
                245   250   255

Glu Tyr Ser Ile Ala Ala Ser Leu Trp Lys Leu Ser Ser Cys Asp Leu
            260   265   270

Cys Glu Ile Ala Arg Asn Ser Val Tyr Gln Ser Gly Phe Ser His Ala
        275   280   285

Leu Lys Ala His Trp Ile Gly Lys Asn Tyr Tyr Lys Arg Gly Pro Ser
    290   295   300

Gly Asn Asp Ile His Arg Thr Asn Val Pro Thr Ile Arg Ile Glu Phe
305   310   315   320

Arg Asp Leu Ile Trp Arg Asp Glu Met Gln Leu Val Tyr Leu Asn Asn
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Glu Leu Ala Lys Thr Asn Asn Ile Ala Leu Pro Asp Ser Ala Ala Asp
        35                  40                  45

Ala Ser Phe Lys Ser Pro Gln Glu Leu Glu Ser Arg Tyr Glu Arg Phe
    50                  55                  60

Thr Ser Leu Asn Asp Phe Leu His Tyr Tyr Tyr Ile Gly Met Ser Val
65                  70                  75                  80

Leu Ile Asn Pro Ala Asp Tyr Glu Ser Leu Ala Tyr Glu Tyr Leu Thr
                85                  90                  95

Lys Ala Asn Arg Asp Gly Val His His Ala Glu Ile Phe Phe Asp Pro
            100                 105                 110

Gln Ala His Thr Glu Arg Gly Ile Ala Tyr Asn Thr Val Val Glu Gly
        115                 120                 125

Leu Ser Ala Gly Leu Lys Arg Ala Glu Lys Asp Phe Gly Ile Thr Ser
    130                 135                 140

Lys Leu Ile Leu Cys Phe Leu Arg His Leu Ser Ala Glu Asp Ala Lys
145                 150                 155                 160

Thr Thr Tyr Gln Glu Ala Val Ser Leu Gly His Phe Ser Asn Gly Thr
                165                 170                 175

Val Ala Ala Ile Gly Leu Asp Ser Ser Glu Val Gly Phe Pro Pro Glu
            180                 185                 190
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Ile Phe Arg Glu Ile Tyr Glu Ser Ala Glu Thr Lys Gly Ile His Arg
195 200 205

Thr Ala His Ala Gly Glu Glu Gly Asp Thr Ser Tyr Ile Ser Arg Ala
210 215 220

Leu Asp Ile Cys Lys Val Glu Arg Ile Asp His Gly Ile Arg Leu Ala
225 230 235 240

Glu Asp Glu Asn Leu Leu Lys Arg Val Ala Glu Gln Gly Thr Met Leu
245 250 255

Thr Val Cys Pro Leu Ser Asn Val Arg Leu Arg Cys Val Glu Asn Val
260 265 270

Gly Gln Leu Pro Ile Arg Lys Phe Leu Asp Gly Gly Ile Lys Phe Ser
275 280 285

Ile Asn Ser Asp Asp Pro Ala Tyr Phe Gly Gly Tyr Ile Leu Asp Asn
290 295 300

Tyr Leu Ala Val Gln Glu Ala Phe Gly Leu Asn Leu Lys Glu Trp Lys
305 310 315 320

Tyr Ile Ala Thr Ser Ala Ile Glu Gly Ser Trp Cys Asp Asp Glu Arg
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Ala Leu Leu
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Thr Val Ala Pro Trp Glu Lys Glu Val Ile Ser Asp Pro Ser Thr Pro
35 40 45

Lys Pro Asn Thr Glu Pro Phe Ala His Tyr Pro Gln Gly Lys Ser Asp
50 55 60

His Cys Phe Glu Met Gln Asp Gly Val Val His Val Phe Ala Asn Lys
65 70 75 80

Asp Ala Lys Glu Asp Leu Phe Pro Val Ala Asp Ala Thr Ala Phe Phe
85 90 95

Thr Asp Leu His His Val Leu Lys Val Ile Ala Ala Gly Asn Ile Arg
100 105 110

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Thr Leu Cys His Arg Arg Leu Val Leu Leu Glu Gln Lys Phe Asn Leu
        115                 120                 125

His Leu Met Leu Asn Ala Asp Lys Glu Phe Leu Ala Gln Lys Ser Ala
    130                 135                 140

Pro His Arg Asp Phe Tyr Asn Val Arg Lys Val Asp Thr His Val His
145                 150                 155                 160

His Ser Ala Cys Met Asn Gln Lys His Leu Leu Arg Phe Ile Lys Ser
                165                 170                 175

Lys Leu Arg Lys Glu Pro Asp Glu Val Val Ile Phe Arg Asp Gly Thr
            180                 185                 190

Tyr Leu Thr Leu Arg Glu Val Phe Glu Ser Leu Asp Leu Thr Gly Tyr
        195                 200                 205

Asp Leu Asn Val Asp Leu Leu Asp Val His Ala Asp Lys Ser Thr Phe
    210                 215                 220

His Arg Phe Asp Lys Phe Asn Leu Lys Tyr Asn Pro Cys Gly Gln Ser
225                 230                 235                 240

Arg Leu Arg Glu Ile Phe Leu Lys Gln Asp Asn Leu Ile Gln Gly Arg
                245                 250                 255

Phe Leu Gly Glu Ile Thr Lys Gln Val Phe Ser Asp Leu Glu Ala Ser
            260                 265                 270

Lys Tyr Gln Met Ala Glu Tyr Arg Ile Ser Ile Tyr Gly Arg Lys Met
        275                 280                 285

Ser Glu Trp Asp Gln Leu Ala Ser Trp Ile Val Asn Asn Asp Leu Tyr
    290                 295                 300

Ser Glu Asn Val Val Trp Leu Ile Gln Leu Pro Arg Leu Tyr Asn Ile
305                 310                 315                 320

Tyr Lys Asp Met Gly Ile Val Thr Ser Phe Gln Asn Ile Leu Asp Asn
                325                 330                 335

Ile Phe Ile Pro Leu Phe Glu Ala Thr Val Asp Pro Asp Ser His Pro
            340                 345                 350

Gln Leu His Val Phe Leu Lys Gln Val Val Gly Phe Asp Leu Val Asp
        355                 360                 365

Asp Glu Ser Lys Pro Glu Arg Arg Pro Thr Lys His Met Pro Thr Pro
    370                 375                 380

Ala Gln Trp Thr Asn Ala Phe Asn Pro Ala Phe Ser Tyr Tyr Val Tyr
385                 390                 395                 400

Tyr Cys Tyr Ala Asn Leu Tyr Val Leu Asn Lys Leu Arg Glu Ser Lys
                405                 410                 415

Gly Met Thr Thr Ile Thr Leu Arg Pro His Ser Gly Glu Ala Gly Asp
            420                 425                 430
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Ile Asp His Leu Ala Ala Thr Phe Leu Thr Cys His Ser Ile Ala His
435 440 445

Gly Ile Asn Leu Arg Lys Ser Pro Val Leu Gln Tyr Leu Tyr Tyr Leu
450 455 460

Ala Gln Ile Gly Leu Ala Met Ser Pro Leu Ser Asn Asn Ser Leu Phe
465 470 475 480

Leu Asp Tyr His Arg Asn Pro Phe Pro Val Phe Phe Leu Arg Gly Leu
485 490 495

Asn Val Ser Leu Ser Thr Asp Asp Pro Leu Gln Ile His Leu Thr Lys
500 505 510

Glu Pro Leu Val Glu Glu Tyr Ser Ile Ala Ala Ser Val Trp Lys Leu
515 520 525

Ser Ala Cys Asp Leu Cys Glu Ile Ala Arg Asn Ser Val Tyr Gln Ser
530 535 540

Gly Phe Ser His Ala Leu Lys Ser His Trp Ile Gly Lys Asp Tyr Tyr
545 550 555 560

Lys Arg Gly Pro Asp Gly Asn Asp Ile His Lys Thr Asn Val Pro His
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20 25 30

Ile Ser Leu Pro Ala Gln Ser Leu Glu Thr Leu Ile Pro His Val Gln
35 40 45

Val Ile Ala Asn Glu Pro Asp Leu Val Ser Phe Leu Thr Lys Leu Asp
50 55 60

Trp Gly Val Lys Val Leu Ala Ser Leu Asp Ala Cys Arg Arg Val Ala
65 70 75 80

Phe Glu Asn Ile Glu Asp Ala Ala Arg His Gly Leu His Tyr Val Glu
85 90 95

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Leu Arg Phe Ser Pro Gly Tyr Met Ala Met Ala His Gln Leu Pro Val
            100                 105                 110

Ala Gly Val Val Glu Ala Val Ile Asp Gly Val Arg Glu Gly Cys Arg
        115                 120                 125

Thr Phe Gly Val Gln Ala Lys Leu Ile Gly Ile Met Ser Arg Thr Phe
    130                 135                 140

Gly Glu Ala Ala Cys Gln Gln Glu Leu Glu Ala Phe Leu Ala His Arg
145                 150                 155                 160

Asp Gln Ile Thr Ala Leu Asp Leu Ala Gly Asp Glu Leu Gly Phe Pro
                165                 170                 175

Gly Ser Leu Phe Leu Ser His Phe Asn Arg Ala Arg Asp Ala Gly Trp
            180                 185                 190

His Ile Thr Val His Ala Gly Glu Ala Ala Gly Pro Glu Ser Ile Trp
        195                 200                 205

Gln Ala Ile Arg Glu Leu Gly Ala Glu Arg Ile Gly His Gly Val Lys
    210                 215                 220

Ala Ile Glu Asp Arg Ala Leu Met Asp Phe Leu Ala Glu Gln Gln Ile
225                 230                 235                 240

Gly Ile Glu Ser Cys Leu Thr Ser Asn Ile Gln Thr Ser Thr Val Ala
                245                 250                 255

Glu Leu Ala Ala His Pro Leu Lys Thr Phe Leu Glu His Gly Ile Arg
            260                 265                 270

Ala Ser Ile Asn Thr Asp Asp Pro Gly Val Gln Gly Val Asp Ile Ile
        275                 280                 285

His Glu Tyr Thr Val Ala Ala Pro Ala Ala Gly Leu Ser Arg Glu Gln
    290                 295                 300

Ile Arg Gln Ala Gln Ile Asn Gly Leu Glu Met Ala Phe Leu Ser Ala
305                 310                 315                 320

Glu Glu Lys Arg Ala Leu Arg Glu Lys Val Ala Ala Lys
                325                 330
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PA ENT COOPERATION TREA

RECEIVED

FEB 28 2001

FEB 27 2001

PATENT RECORDS CENTER

From the INTERNATIONAL SEARCHING AUTHORITY

To:

E.I. DU PONT DE NEMOURS AND COMPANY
Legal/Patent Records Center
Attn. KENING, Li
1007 Market Street
Wilmington, Delaware 19898
UNITED STATES OF AMERICA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 22/02/2001
Applicant's or agent's file reference BB1386 PCT	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 00/ 21009	International filing date *(day/month/year)* 28/07/2000
Applicant E.I. DU PONT DE NEMOURS AND COMPANY	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO
   34, chemin des Colombettes
   1211 Geneva 20, Switzerland
   Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

   Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

   Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heike Zoglauer

REY NOTED

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]: "Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]: "Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]: "Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or "Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]: "Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

P. ENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BB1386 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/21009	28/07/2000	30/07/1999
Applicant		
E.I. DU PONT DE NEMOURS AND COMPANY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/21009

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24 all partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:1, 2, 13 and 14 that correspond to a Zea mays AMP deaminase, methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

2. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:3, 4, 15 and 16 that correspond to an Oryza sativa AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

3. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:5, 6, 17 and 18 that correspond to a Glycine max AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

4. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:7, 8, 19 and 20 that correspond to a Triticum aestivum AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/21009

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/78 C12N15/52 C12N15/80 C12N15/82 C12N15/10
C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE NCBI [Online] GI = 7484807, AMP deaminase homolog, 14 May 1999 (1999-05-14) ROUNSLEY ET AL.: "AMP deaminase homolog F16M14.21 - Arabidopsis thaliana" XP002150377 cited in the application the whole document	1-23
Y		24

X	DATABASE EMBL [Online] Acc. Num. AI731574, putative deaminase, 12 June 1999 (1999-06-12) BLEWITT ET AL.: "ESTs from developing cotton fiber" XP002150378 the whole document	1-23
Y		24

	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2000	22.02.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Burkhardt, P

3

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 00/21009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DANCER ET AL: "Adenosine-5'-phosphate deaminase" PLANT PHYSIOLOGY,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD,US, vol. 114, 1997, pages 119-129, XP002110870 ISSN: 0032-0889 the whole document -----	24

3

14

PATENT COOPERATION TREATY

REC'D 29 NOV 2001
PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BB1386 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/21009	International filing date *(day/month/year)* 28/07/2000	Priority date *(day/month/year)* 30/07/1999
International Patent Classification (IPC) or national classification and IPC C12N15/00		
Applicant E.I. DU PONT DE NEMOURS AND COMPANY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/02/2001	27.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Burkhardt, P Telephone No. +49 89 2399 7456

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

   Description, pages:

   1-32 as originally filed

   Claims, No.:

   1-24 as originally filed

   Drawings, sheets:

   1/4-4/4 as originally filed

   Sequence listing part of the description, pages:

   1-31, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1 - 24 (all partially).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 5, 6, 9, 16 - 24

	No:	Claims	1 - 4, 7, 8, 10 - 15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	5, 6, 9, 16 - 24
Industrial applicability (IA)	Yes:	Claims	1 - 24
	No:	Claims	

2. Citations and explanations
   see separate sheet

Re Item IV

Lack of unity of invention

The ISA found a lack of unity with the present set of claims. The applicant did not reply to the invitation to pay additional fees. The search was therefore limited to invention 1 (claims 1 - 24, all partially) relating to SEQ ID NOs:1, 2, 13 and 14 that correspond to a putative *Zea mays* AMP deaminase, methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

Consequently, the written opinion will also be limited to invention 1, i.e. to claims 1 - 24 (all partially) relating to SEQ ID NOs:1, 2, 13 and 14.

Re Item V

Reasoned statement under Article 35 with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure and is following the order of the International Search Report:

D1 NCBI Online, GI = 7484807 (Rounsley *et al.*, 1999)
D2 EMBL Online, Acc. No. AI731574 (Blewitt *et al.*, 1999)
D3 Dancer *et al.*, 1997 Plant Physiol. 114:119-129.

1. Statement of invention

1.1 The applicants have isolated and sequenced a *Zea mays* polynucleotide which has a certain identity to known plant AMP deaminases. The analysis of the postulated deaminase function is not provided as experimental data supporting the function are not part of the application.

2. Article 33(2)PCT (Novelty)

2.1 D1 discloses an isolated polynucleotide encoding a polypeptide that shows 100% identity to SEQ ID NOs:2 and 14 in stretches of up to 50 amino acids. D2 discloses an isolated polynucleotide encoding a polypeptide that shows more than 90% identity in stretches of more than 35 amino acids to the above SEQ ID NOs. D1 and D2 thus anticipate the subject-matter of present claim 1, a polynucleotide encoding a polypeptide of at least 35 amino acids that has at least 85% identity to SEQ ID NOs:2 or 14. The same holds true for dependent claims 2 - 4, 7 and 8 as well as for claims 10 - 15. Said claims do not meet the requirements of Article 33(2) PCT.

3. Article 33(3) PCT (Inventive step)

3.1 Present claims 5, 6, 9 and 16 - 23 relate to subject-matter that is not novel (see above). It does not involve an inventive step to employ known polynucleotide sequences for DNA constructs, plant transformation and the like. Methods for these purposes are well known and a man skilled in the art would choose them according to his needs. Claims 5, 6, 9 and 16 - 23 do not meet the requirements of Article 33(3) PCT.

3.2 A similar objection applies to present claim 24. Methods for evaluating compounds for their ability to inhibit the activity of an AMP deaminase are known from D3 (page 119, Material and Methods). It does not involve an inventive step to transfer such methods to cells that have been transformed with an AMP deaminase. Claim 24 does therefore not meet the requirements of Article 33(3) PCT.

3.3 If the applicant succeeds in establishing formal novelty for the subject-matter of present claim 1 he is requested to consider the following:

3.4 The applicant isolated and sequenced a polynucleotide from Zea mays. However, no functional analysis was provided.
Nucleotide sequences From *Z. mays* are known in the prior art. The technical

problem may thus be formulated as the provision of further nucleotide sequences from *Z. mays*.

3.5 An arbitrary choice of a nucleotide sequence from *Z. mays* cannot involve an inventive step. In order to be patentable, a selection must not be arbitrary but must be justified by the technical purpose, i.e. by a hitherto unknown or unexpected technical effect which is caused by those structural features distinguishing the nucleotide sequence from the numerous other ones. However, this is not the case for SEQ ID NO:1,2,13 or 14, respectively. Therefore, present claim 1 and claims 2 - 24 depending or relating on the subject-matter of claim 1 do not meet the requirements Article 33(3) PCT.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/09305 A3

(51) International Patent Classification[7]: C12N 9/78, 15/52, 15/80, 15/82, 15/10, 5/10, A01H 5/00

(21) International Application Number: PCT/US00/21009

(22) International Filing Date: 28 July 2000 (28.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/146,473 30 July 1999 (30.07.1999) US

(71) Applicants *(for all designated States except US)*: E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 7100 N.W. 62nd Avenue, Johnston, IA 50131 (US).

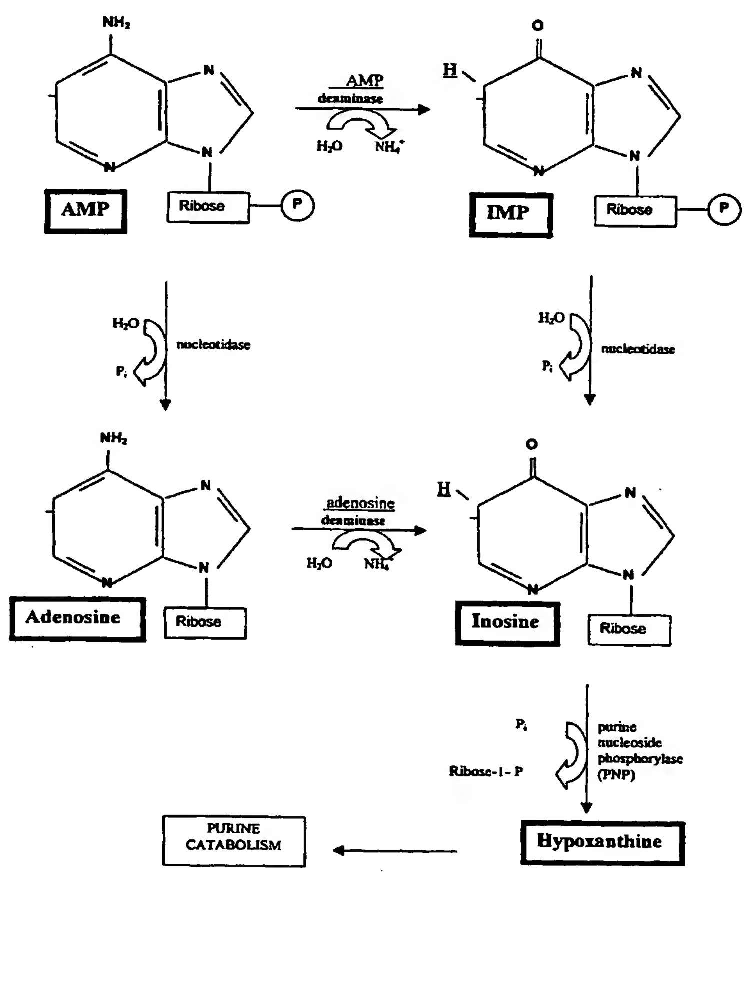
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CASPAR, Timothy [US/US]; 2927 Barley Mill Road, Yorklyn, Wilmington, DE 19736 (US). FALCO, Saverio, Carl [US/US]; 1902 Miller Road, Arden, DE 19810 (US). SAKAI, Hajime [DE/US]; 105 Banbury Drive, Wilmington, DE 19803 (US). WENG, Zude [CN/US]; Apartment 301, 495 Leslie Court, Des Plaines, IL 60016 (US). HU, Xu [CA/US]; 4700 103rd Street, Urbandale, IA 50322 (US).

(74) Agent: LI, Kening; E.I. Du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO,

[Continued on next page]

(54) Title: PURINE METABOLISM GENES IN PLANTS



(57) Abstract: This invention relates to an isolated nucleic acid fragment encoding an AMP deaminase or adenosine deaminase, a transformed host cell comprising the nucleic acid fragment, and a transgenic plant comprising the nucleic acid fragment. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of an AMP or adenosine deaminase, and a chimeric gene comprising the isolated fragment in sense or antisense orientation. This invention further relates to a method for altering expression level of AMP deaminase or adenosine deaminase in a transformed host cell, and a method for evaluating a compound that affects the activity of an AMP deaminase or an adenosine deaminase.

WO 01/09305 A3

RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) **Date of publication of the international search report:** 13 September 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/21009

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/78 C12N15/52 C12N15/80 C12N15/82 C12N15/10
C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE NCBI [Online] GI = 7484807, AMP deaminase homolog, 14 May 1999 (1999-05-14) ROUNSLEY ET AL.: "AMP deaminase homolog F16M14.21 - Arabidopsis thaliana" XP002150377 cited in the application the whole document	1-23
Y		24

X	DATABASE EMBL [Online] Acc. Num. AI731574, putative deaminase, 12 June 1999 (1999-06-12) BLEWITT ET AL.: "ESTs from developing cotton fiber" XP002150378 the whole document	1-23
Y		24

	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2000	22.02.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Burkhardt, P

3

Form PCT/ISA/210 (second sheet) (July 1992)

Inte onal Application No
PCT/US 00/21009

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DANCER ET AL: "Adenosine-5'-phosphate deaminase" PLANT PHYSIOLOGY, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US, vol. 114, 1997, pages 119-129, XP002110870 ISSN: 0032-0889 the whole document -----	24

3

In ational application No.
PCT/US 00/21009

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24 all partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:1, 2, 13 and 14 that correspond to a Zea mays AMP deaminase, methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

2. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:3, 4, 15 and 16 that correspond to an Oryza sativa AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

3. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:5, 6, 17 and 18 that correspond to a Glycine max AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

4. Claims: Claims 1 - 24 (all partially)

Claims 1 - 24 (all partially) relating to SEQ ID NOs:7, 8, 19 and 20 that correspond to a Triticum aestivum AMP deaminase, to methods of obtaining said deaminase, to methods of altering the expression level of said deaminase by transforming host cells, to the transformed host cells and to methods of evaluating inhibitors of said AMP deaminase.

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PCT/US00/21009

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 May 2001 (07.05.01)	
International application No. PCT/US00/21009	**Applicant's or agent's file reference** BB1386 PCT
International filing date (day/month/year) 28 July 2000 (28.07.00)	**Priority date** (day/month/year) 30 July 1999 (30.07.99)
Applicant CASPAR, Timothy et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 February 2001 (21.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Nestor Santesso Telephone No.: (41-22) 338.83.38